Role of Bio- fertilizer and Amino acid spraying on growth and yield of two type Mint plant and its contents of some active constituents

Zahraa Fadhil Hussein Al-Yasiry Abbas Khdiar Majwel

College of Agriculture ,Al-Qasim Green University

Abstract

The experiment was coundected on one of the private farms in the Al-Azzawiya area / Al-Musayyib district for the spring season 2022-2023 in Babylon province. Within the Randomized Complete Block Design (RCBD) in a split-split plot system arrangement with three replicates, where each replicate contains 18 treatments. The experiment included 54 experimental units. Biofertilizer was placed in the sub plots (Split-Split Plot System) and amino acids were placed in sub plot and cultivar represents the main plot (Main-plot). The experiment included three factors as follows: the first factor: two cultivar of mint plot (Mentha Longfolia, Mentha Spicata) and the second factor: spraying amino acids (comparison without spraying, spraying phenylalanine acid at a concentration of 150 mg. L-1, spraying tyrosine acid with a concentration of 150 mg. L-1. The third factor: adding biofertilizer, which includes three levels (control without fertilization, Mycorrhiza (Glomus mosseae) fertilizer with a concentration of 5 g , Azotobacter chroococcum fertilizer with a concentration of 5 g) and it was compared. Average coefficients using the least significant difference (L.S.D.) at the probability level of 0.05. The results can be summarized as follows:

The Mentha Longfolia plant showed a significantly excelled in plant height, number of branches, ascorbic acid, and percentage of oil. The mint plant also showed a significantly excelled in the percentage of limonene %, phenylalanine also gave a superiority in the percentage of oil %, and tyrosine acid gave a significantly excelled in the percentage of oil. Plant, nitrogen%, phosphorus%, ascorbic acid, and limonene%. The data showed superiority to the biofertilizer (the mycorrhizal fungus Glomus mosseae) in the number of branches, phosphorus%, and limonene%. The biofertilizer (Azotobacter chroococcum bacteria) also showed a significantly excelled in plant height, nitrogen percentage, and ascorbic acid. The binary interaction between Mentha Longfolia and tyrosine showed a significantly excelled in plant height, nitrogen percentage, and ascorbic acid. As for mint with tyrosine, it was superior in the limonene percentage. Also, the binary interaction of Mentha Longfolia cultivar and phenylalanine acid showed a significant increase in phosphorus percentage and oil percentage. The interaction treatment of Mentha Longfolia with Azotobacter bacteria achieved significant improvement in plant height, number of branches, percentage of nitrogen, ascorbic acid, and percentage of oil. The interaction of Mentha Longfolia with mycorrhizal fungi showed an increase in the percentage of phosphorus. As for the interaction of mint plants with the mycorrhizal fungus, it achieved superiority in the percentage of limonene%. The interaction of tyrosine acid with the mycorrhizal fungus gave a significantly excelled in the percentage of phosphorus% and limonene%. The interaction of phenylaniline acid with Azotobacter bacteria achieved a significantly excelled in terms of plant height, nitrogen percentage, and acidity. Ascorbic acid and the same acid with mycorrhizal fungi gave a significantly excelled in oil percentage, and the triple interaction of Mentha Longfolia with tyrosine acid and Azotobacter bacteria achieved a

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significantly excelled in nitrogen percentage, and the triple interaction of Mentha Longfolia with tyrosine acid with mycorrhizal fungi gave a superiority in phosphorus percentage. As for the triple interaction of Mentha Longfolia with phenylalanine and Azotobacter bacteria, there was a significant increase in ascorbic acid and percentage of oil.
Keywords: Glomus mossea, Azotobacter chroococcum, Mentha Longfolia, biofertilizer

Introduction

Medicinal plants are an important source of medicines and play an important role in the global health system. Therefore, today it has become important to the global economy [22]. Its parts contain aromatic or volatile oils [23]. They are called essential oils or aromatic oils because of their distinctive smell, and they are called ether oils because of their ability to dissolve in ether. It also has the advantage of being volatile at high temperatures and does not decompose unlike fixed oils [7]. Therefore, it is considered an important source of medicines that play an important role in the global health system. Therefore, today it has become important for the global economy [22.[

Amino acids are nitrogenous organic compounds, where they are considered the basic unit in building proteins, which contribute to maintaining the stability of cell acidity, as well as in the formation of amides that remove ammonia from the cell, in addition to being a source of carbon and energy. They protect plants from pathogens, where they contribute to overcoming nutritional stress during pregnancy. The life cycle of plants increases the protein content of the tissues by building new types of proteins and enzymes necessary to regulate the metabolic activities of plants [11]. (The growth of plants and their production of secondary compounds is affected by many factors, including the spraying of amino acids, which are the initiators of the formation of hormones and growth regulators. They are also considered biostimulants. Therefore, it has a significant impact on the chemical and qualitative compounds of plants [13].The use of biofertilizers has increased at the present time, which is one of the important technologies that have been introduced in the field of agriculture to increase production and improve quality, where the beneficial microorganisms present in biofertilizers maintain the ecosystem, improve the chemical, physical and biological properties of the soil, plant growth and increase crop productivity [25]. It also contains a number of microorganisms that differ depending on the purpose for which this fertilizer is used. Biofertilizers can be divided in terms of their nature and behavior in the soil into symbiotic biofertilizers, as they are produced from the activity of microorganisms that live cooperatively with the roots of the daughters, as well as These microbes provide the daughters with some nutrients and take their nutritional needs, especially the source of carbon from the daughters. This means that there is a mutual benefit between two different organisms that live together, meaning that they support each other, such as the mycorrhizea fungus, which is used as a biofertilizer for the daughters and the soil for its role in increasing the surface area of the daughter's roots. Which improves the absorption of nutrients from the soil, especially in lands that suffer from a deficiency in nutrients such as phosphorus, thus stimulating the growth of daughters and increasing the efficiency of nutrient absorption. It also tolerates unsuitable environmental conditions and is resistant to pathogens [19.[

The other type is asymbiotic biofertilizers, as it is characterized by the fact that the microorganisms used in its production live freely in the soil and obtain their nutritional needs from the soil, such as Azotobacter. These Azotobacter bacteria are distinguished by their large cells compared to other bacterial species [21]. It is usually spread in light basic soils and neutral soils. It can fix atmospheric nitrogen, in addition to its ability to dissolve phosphate. It produces various types of growth regulators such as indole acetic acid, gibberellins, cytokinins, and a number of vitamins such as (B1 (Thiamin), (B2) Riboflavin) and (B3). Niacin, B12(Cobalamin, B9(Folic acid), Biotin (B7), and Pantothenic acid (B5), which stimulate root growth and the formation of root hairs. Azotobactere also inhibit daughter pathogens and the production of siderophores [18,17.[

Given the abundance of medical facilities in Iraq and the desire to develop the role they play in achieving the basic part of pharmaceutical and therapeutic security for society, I proposed this study, which included aims:

study aim

-1Knowing the effect of biofertilization on vegetative and quantitative growth, chemical indicators, and some active compounds in the oil of two types of mint plants.

-2Knowing the effect of amino acids on traits of vegetative and quantitative growth, chemical indicators, and some active compounds in the oil of two types of mint plants.

-3Knowing the effects of interaction between study factors on vegetative and quantitative growth traits, chemical indicators, and some active compounds.

-Materials and methods

Experiment implementation :

The experiment or research was conducted in an agricultural area in Al-Musayyib district north of Babylon (Al-Azawiyah) during the spring agricultural season of 2023, within longitude 44.07° and latitude 32.76°, to study the role of biofertilization and spraying amino acids in the growth and production of two types of mint plants and their content in some effective compounds. .

Study factors: The experiment included three factors:

The first factor: cultivar, symbolized by V

The first type: Mint cuttings of the Mentha spicata cultivar, which has the symbol V1, were planted

The second cultivar: cuttings of the Mentha Longfolia plant, the symbol is V2.

2- The second factor: amino acids, symbolized by A

: A1 Spray distilled water only

A2: Spraying the amino acid phenylalanine at a concentration of 150 mg.L-1, taken from Direvo Germany.

A3: Spraying the amino acid Tyrosine at a concentration of 150 mg.L-1, taken from Direvo Germany.

I sprayed the plants in the morning until they were completely wet, adding Zahi as a diffuser, at a rate of 1 ml/10 L-1 [4]. The first spraying was on 5/1/2023, 40 days after planting in the field, with three sprayings, and three weeks between one spraying and the next.

B - The third factor: biological fertilization, symbolized by 3

B1: which included the addition of sterile moss only

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B2: It included adding ground to mycorrhizal fungi. Glomus spp, loaded with mycorrhiza on peat moss, and one gram contains -30 35 spores.
The ground was fertilized by mixing the inoculum from the selected isolate of the mycorrhizal fungus. Glomus spp, which is fungus hibiscus and its spores, is taken from infected roots and mixed with sterilized peat moss in an equal proportion, and 5 g are added to each plant per hole during the process of planting mint cuttings in the field. The treatments were applied to the two cultivar of mint plants before planting and on the same day on 3/25/2023.
B3: It included the addition of ground bacteria Azotobacter chroococcum
At a concentration of 310*25 CFU ml-1, at a rate of 5 g per plant, per pit, at the same date of planting.
Mycorrhizal fungi and Azotobacter bacteria were obtained from the laboratories of the Agricultural Research Department - Center for Biotechnology - Ministry of Science and Technology(
5- -3Experimental design:
The experiment was carried out as a factorial experiment (3 × 3 × 2) with a randomized complete block design according to the split plot system and with three replications. The cultivar were distributed on the main plot, the amino acids on the sub-plot, and the biological fertilization on the sub-plot. Sub-sub-plots [8] with three replicates, with 18 experimental units in each replicate, so the number of experimental units is (54.(

Experimental readings and measurements.
The height of six random plants was measured at the end of the season using a metric tape measure from the area of plant contact with the soil (crown area) to the highest growing apex of the plant, then averaged.
Number of branches (branch.plant-1(
The number of lateral plant branches of six plants from each experimental unit was measured and the average was taken for them.
-Percentage of nitrogen(%)
The percentage of total nitrogen was estimated with a Micro Kjeldahl device using the method described by [21[
- Percentage of phosphorus(%)
The percentage of phosphorus in the plant was estimated following the method [9.[
Determination of ascorbic acid (mg.100g-1 fresh weight(
The method used by [27] was used to measure ascorbic acid.
Oil percentage%
The percentage of oil in the ground dry leaf samples was estimated according to [14]. by applying the following equation:
Percentage of oil=(Weight of the produced oil (g))/(sample weight (g))×100
% Limonene
The examination was conducted in the laboratories of the Ministry of Science and Technology / Department of Environment and Water using a gas chromatography (GC) device, model Shimadzu 2010, made in Japan, using a flame detector (FID.(

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Table () of the studied traits

treatments	Plant height(cm)	Number of branches (branch.plant-1)	Nitrogen %	Phosphorus%	Ascorbic acid mg. 100 g $^{-1}$ fresh weight.	Oil%	%Limonen
V1	37.44	8.31	2.103	0.142	32.11	0.900	5.5278
V2	50.65	9.44	2.254	0.162	40.97	1.053	0.8617
L.S.D(V)	1.881	N.S	N.S	0.0184	5.489	0.1084	0.12147
A1	41.28	7.95	1.851	0.128	30.36	0.901	2.8008
A2	45.15	8.93	2.341	0.156	38.97	1.043	3.2567
A3	45.70	9.74	2.344	0.172	40.28	0.985	3.5267
L.S.D(A)	1.577	0.	0.2836	0.0074	1.818	0.0500	0.00996
B1	42.94	8.23	1.824	0.133	31.34	0.919	2.6633
B2	43.32	9.23	2.183	0.169	36.95	1.012	3.6867
B3	45.86	9.17	2.528	0.153	41.31	0.999	3.2342
L.S.D (B)	2.006	0.551	0.2527	0.0101	2.711	0.0846	0.01615
V1A1	35.39	7.59	1.788	0.116	27.25	0.794	5.0367
V1A2	38.55	8.50	2.517	0.156	35.07	0.975	5.6250
V1A3	38.37	8.83	2.003	0.155	34.00	0.932	5.9217
V2A1	47.16	8.31	1.914	0.139	33.46	1.009	0.5650
V2A2	51.74	9.37	2.164	0.169	42.88	1.111	0.8883
V2A3	53.03	10.65	2.684	0.153	46.56	1.039	1.1317

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L.S.D(V.A)	**2.641**	**1.522**	**0.4436**	**0.0142**	**4.242**	**0.0857**	**0.11659**
V1B1	**35.90**	**7.45**	**1.706**	**0.119**	**28.85**	**0.853**	**4.8883**
V1B2	**36.52**	**8.97**	**2.143**	**0.161**	**30.99**	**0.924**	**6.1317**
V1B3	**39.89**	**8.49**	**2.459**	**0.147**	**36.48**	**0.925**	**5.5633**
V2B1	**49.98**	**9.00**	**1.942**	**0.148**	**33.84**	**0.986**	**0.43837**
V2B2	**50.13**	**9.48**	**2.223**	**0.177**	**42.92**	**1.101**	**1.241**
V2B3	**51.83**	**9.85**	**2.598**	**0.160**	**46.15**	**1.072**	**0.9050**
L.S.D(V.B)	**2.481**	**1.442**	**0.4272**	**0.0154**	**4.416**	**0.1117**	**0.10822**
A1B1	**37.90**	**7.48**	**1.192**	**0.112**	**27.37**	**0.735**	**2.4100**
A1B2	**42.25**	**8.41**	**1.873**	**0.136**	**31.46**	**0.914**	**3.2000**
A1B3	**43.67**	**7.97**	**2.488**	**0.134**	**32.24**	**1.059**	**2.7925**
A2B1	**45.72**	**8.07**	**2.032**	**0.137**	**32.16**	**0.998**	**2.5825**
A2B2	**42.66**	**8.80**	**2.373**	**0.183**	**36.95**	**1.172**	**3.8100**
A2B3	**47.05**	**9.94**	**2. 618**	**0.150**	**47.81**	**0.959**	**3.3775**
A3B1	**45.19**	**9.14**	**2.248**	**0.151**	**34.50**	**1.025**	**2.9975**
A3B2	**45.05**	**10.48**	**2.305**	**0.189**	**42.45**	**0.950**	**4.0500**
A3B3	**46.86**	**9.61**	**2.478**	**0.176**	**43.89**	**0.982**	**3.5325**
L.S.D(A.B)	**3.129**	**0.873**	**0.4333**	**0.0156**	**4.118**	**0.1265**	**0.02428**

V.B.A	**Plant height(cm)**	**Number of branches (branch.plant-1)**	**Nitrogen%**	**Phosphorus%**	**Ascorbic acid mg. 100 g $^{-1}$ fresh weight.**	**Oil%**	**%Limonen**

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V1A1B1	**33.75**	**6.94**	**1.130**	**0.105**	**25.28**	**0.634**	**4.5900**
V1A1B2	**35.23**	**8.22**	**1.773**	**0.120**	**27.74**	**0.831**	**5.4750**
V1A1B3	**37.19**	**7.61**	**2.460**	**0.124**	**28.74**	**0.918**	**5.0450**
V1A2B1	**37.11**	**7.64**	**2.152**	**0.115**	**30.40**	**0.958**	**4.8250**
V1A2B2	**37.39**	**8.54**	**2.630**	**0.198**	**30.64**	**1.027**	**6.3450**
V1A2B3	**41.16**	**9.32**	**2.770**	**0.156**	**44.15**	**0.939**	**5.7050**
V1A3B1	**36.83**	**7.77**	**1.837**	**0.137**	**30.87**	**0.966**	**5.2500**
V1A3B2	**36.94**	**10.16**	**2.027**	**0.166**	**34.59**	**0.912**	**6.5750**
V1A3B3	**41.33**	**8.55**	**2.147**	**0.161**	**36.54**	**0.917**	**5.9400**
V2A1B1	**42.05**	**8.02**	**1.253**	**0.120**	**29.45**	**0.836**	**0.2300**
V2A1B2	**49.27**	**8.59**	**1.972**	**0.153**	**35.19**	**0.997**	**0.9250**
V2A1B3	**50.16**	**8.33**	**2.517**	**0.145**	**35.74**	**1.193**	**0.5400**
V2A2B1	**54.34**	**8.50**	**1.912**	**0.159**	**33.92**	**1.038**	**0.3400**
V2A2B2	**47.94**	**9.05**	**2.115**	**0.168**	**43.25**	**1.317**	**1.2750**
V2A2B3	**52.94**	**10.55**	**2.467**	**0.144**	**51.46**	**0.978**	**1.0500**
V2A3B1	**53.55**	**10.50**	**2.660**	**0.165**	**38.13**	**1.083**	**0.7450**
V2A3B2	**53.16**	**10.80**	**2.583**	**0.211**	**50.31**	**0.988**	**1.5250**
V2A3B3	**52.39**	**10.66**	**2.810**	**0.191**	**51.24**	**1.046**	**1.1250**
L.S.D (V.B.A)	**4.366**	**1.581**	**0.6341**	**0.0232**	**6.290**	**0.1604**	**0.09534**

It is noted from the results presented in the experiment tables that the cultivar had a significant effect on the vegetative and chemical indicators, as it excelled in the following studied traits (plant height, number of branches, phosphorus

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percentage, ascorbic acid), respectively. The reason for the superiority in traits between the cultivar may be attributed to the difference in The hereditary behavior that genes follow in their influence on biological and physiological processes, which are responsible for growth in one cultivar rather than another, and which is reflected positively in traits of vegetative growth. Or the reason may be the superiority of the cultivar in vegetative traits, which was reflected positively in the qualitative indicators. The difference in the performance of the genotypes, which is due to the genetic factors specific to the cultivar and their influence on environmental factors [1] may have led to the superiority of one cultivar over another. As for the mint plant, it excels in the active ingredient Limonen.%

As for adding amino acids to the plant, it had a significant effect on increasing the plant's content of elements, especially nitrogen, which in turn led to an increase in the shoots. On the other hand, spraying amino acids plays a role in changing the osmotic potential in the plant tissue, as it reduces the water potential, which leads to an increase The ability of cells to absorb water and elements, and this has a positive effect on increasing vegetative growth and thus increasing nutrient absorption. Tyrosine acid achieved significantly excelled in vegetative, quantitative and chemical traits (plant height, number of branches, nitrogen percentage, phosphorus percentage, ascorbic acid, limonene%), respectively. The reason for the increase in vegetative growth traits when treated with the amino acid tyrosine can be It may be through the regulatory effects of amino acids, as they affect plant development through their effect on the biosynthesis of the gibberellin hormone. They also immediately provide plant cells with a source of organic nitrogen, which can be taken up by the cells more quickly than inorganic nitrogen. The reason may be due to the fact that some compounds They are affected by amino acids and are called plant growth substances. They are generally similar to hormones in their action, as they control the use of nutrients for the coordinated and balanced development of the plant body, which affects the growth traits of the plant. The excelled of tyrosine acid in vegetative traits may be reflected in the chemical traits, as Tyrosine acid works in the formation of Phenolic compounds and their derivatives, which participate by 60% in the production of polyphenolic compounds (flavonoids) and by 30% in the production of phenolic acids, which ultimately leads to an increase in phenolic compounds and thus to an increase in the percentage of active substances in plant oil [20]. This agreed with [2]on sage plants when sprayed with tyrosine acid.As for adding the amino acid phenylalanine, it achieved a significant increase in the percentage of total oil %. The reason is attributed to the role of amino acids, which are considered the basic building blocks for building proteins, which is reflected in the construction of cell protoplasm and the formation of plant tissues. The amino acid phenylalanine also has an important biological role in many functions. Physiological functions, such as regulating metabolism, transporting and storing nitrogen, and amino acids are important in stimulating cell growth by providing them with nitrogen, carbon and energy to carry out vital activities (Nahed et al., 2010). The

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increase in these indicators may be due to the importance of Phenylalanine in building gibberellic acid by providing the basic building material. The vital component of gibberellin is the organic acid acetate, as the catabolism of the amino acid phenylalanine ends with the formation of acetate and fumarate (Tamboli, 2012), and this was reflected positively in the chemical and quantitative traits of [10]. These results agreed with [29] on the mint plant and [26] on the dill plant.The mycorrhizal fungus also had a significant effect on the vegetative, quantitative and chemical growth traits (number of branches, % phosphorus, % lemon) and this is attributed to the role of the mycorrhizal fungus in converting organic phosphorus into mineral by secreting the phosphatase enzyme so that it can be transferred to the plant by fungal hyphae. It is an element Phosphorus is one of the necessary elements for the plant for its direct role in all vital processes, such as cell division and elongation, transferring energy to all parts of the plant, and increasing the efficiency of photosynthesis to release the energy needed for vital processes within the plant. It is involved in the synthesis of nucleic and amino acids, all of which affect the increase of most vegetative traits [9].). The reason may be due to the role played by mycorrhizae, as one of the most important goals of inoculation with mycorrhizal fungi is to increase the absorption of phosphorus from soils poor in this element, by increasing the surface area for absorption. In addition, the occurrence of infection and colonization of the fungus in the root tissue may help activate proton pumping enzymes. H+ATPase by inducing the genes LHA4, LHA1, and LHA for this enzyme that helps transport phosphorus into cells. This is consistent with what was indicated by [3,12,16,24.[

The mycorrhizal fungus is considered a system that modifies soil fertility through the extension of fungal hyphae to long distances in the soil through the root capillaries, increasing the area of absorption and thus increasing the absorption of nutrients as well as water from the soil solution. In addition, infecting the roots with mycorrhizal fungi leads to an increase in plastids, chlorophyll and activity. Mitochondria improve the nutritional status of the plant, and vegetative growth improves, especially the leaf area of the plant, and this is reflected positively in traits of the crop [6] Mycorrhizae are also characterized by the presence of a network of hyphae around the root system of the host tissues, called extraradical mycelium, which search for water and mineral nutrients in The soil provides plants with these requirements, including fungal hyphae, or they may penetrate the cells of plant roots, forming fine, branching structures called arbuscules within the cells of the root cortex, whose function is to exchange nutrients between the fungi and the plant cells. In addition, some types of mycorrhizae form enlarged structures at the ends of the internodes located inside the tissue cells of plant roots, called vesicles, which transform into storage organs for fats and carbohydrates obtained from the products of photosynthesis in the leaves of the host plant [28.[

As for adding the biofertilizer Azotobacter bacteria to the plant, it had a significant effect on increasing the vegetative, quantitative and qualitative traits (plant

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height, nitrogen percentage, ascorbic acid, oil percentage), respectively. The reason for this may be attributed to the Azotobacter bacteria secreting chelated compounds for several Nutrients such as phosphorus, iron, manganese, and zinc, which ultimately lead to increasing the plant's nutrient content, in addition to the participation of some of them as enzyme cofactors in a number of reactions related to respiration (TCA) and energy transfer (ETC), and the ability of beneficial microorganisms to stimulate the growth of some crops by producing compounds. Nutrient chelator. Also, these bacteria are able to provide some vitamins such as Tiamin B1, Riboflavin B2, Pyridoxine B6, and Cobalamin B12, and some organic acids such as Nicotinic acid, Hetroaxin, Folic acid, and Patothenic acid, which stimulate vegetative growth, in addition to their ability to secrete biochemical compounds. Such as auxins, gibberellins, and cytokinins, which are known for their role in cell division and cell elongation, which is directly reflected in vegetative growth indicators, in addition to improving the growth of the root system by increasing cell division and elongation, which works to increase the surface area of the roots and thus increases the absorption of nutrients, and increased shoot growth is a result The effect of the Azotobacter biofertilizer, which led to an increase in the accumulation of dry matter resulting from an increase in the pace of photosynthesis processes that mainly produce primary metabolic compounds, which in turn led to an increase in the accumulation of intermediate compounds that participate in secondary metabolism to produce phenolic compounds, flavonoids, and others. Also, the speed of photosynthesis processes has increased It created an increase in the biological system's need for compounds and preserved enzymatic reactions in building dry matter, which increased the concentrations of ascorbic acid, which plays an important basic role in the plant's vital activities [5]. This is what was agreed with[15,26]. The significantly excelled of the interaction between the factors, whether bi- or triple interaction, is the result of the participation of the study factors.

Conclusions:

-1The response of the Mentha Longfolia plant and its excelled in most vegetative and chemical traits.

-2Fertilization with mycorrhizal fungi was significantly excelled on all active substances in the oil.

-3When fertilized, Azotobacter bacteria showed significantly excelled in quantitative and qualitative traits related to oil.

-4The interaction between the experimental factors had a positive impact on increasing the content and quality of the volatile oil.

-5The best interaction in oil percentage and total weight was achieved by spraying with phenylalanine acid and fertilizing with mycorrhizal fungus of Mentha longfolia.

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